

## CLAIMS

What is claimed is:

1. A method of generating multiple copies of a nucleic acid sequence of  
5 interest, said method comprising the steps of:

(a) hybridizing a composite primer to a target polynucleotide, wherein the  
composite primer comprises an RNA portion and a 3' DNA portion, the 3' DNA  
portion comprising a 3' most nucleotide, such that the 3' most nucleotide of the 3'  
10 DNA portion hybridizes from about 1 to about 10 nucleotides from the sequence of  
interest;

(b) extending the composite primer with DNA polymerase under conditions  
that permit primer extension, whereby a primer extension product is produced; and  
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(c) cleaving the RNA portion of the primer extension product of (b) with an  
enzyme that cleaves RNA from an RNA/DNA hybrid such that the cleaved primer  
extension product dissociates from the target polynucleotide,

20 wherein the primer extension product is of a size that when the RNA is cleaved the  
cleaved primer extension product dissociates from the target polynucleotide under  
essentially the same conditions as those for primer extension, whereby multiple  
copies of the sequence of interest are produced.

25 2. The method of claim 1, wherein said conditions that permit primer  
extension comprise at least one terminator deoxyribonucleotide triphosphate or  
analog thereof.

3. The method of claim 1, wherein said conditions that permit primer extension comprise the absence of a nucleotide that must be provided for primer extension to continue.

5           4. The method of claim 1, wherein the 3' most nucleotide of the 3' DNA portion of the composite primer hybridizes 1 nucleotide from the sequence of interest.

5. The method of claim 4, wherein the conditions for primer extension comprise one terminator deoxyribonucleotide triphosphate or analog thereof.

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6. The method of claim 1, wherein the sequence of interest is a single nucleotide base.

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7. The method of claim 1, wherein the sequence of interest is more than a single nucleotide base.

8. The method of claim 1, wherein the RNA portion of the composite primer is 5' with respect to the 3' DNA portion.

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9. The method of claim 8, wherein the 5' RNA portion is adjacent to the 3' DNA.

10. The method of claim 1, wherein the enzyme that cleaves RNA is RNase H.

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11. The method of claim 1, wherein the DNA polymerase lacks strand displacement activity.

12. The method of claim 1, wherein the target polynucleotide is DNA or cDNA.

13. The method of claim 1, wherein the target polynucleotide is amplified.

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14. The method of claim 13, wherein amplification occurs simultaneously with generating multiple copies of a sequence of interest.

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15. The method of claim 13, wherein amplification occurs prior to generating multiple copies of a sequence of interest.

16. A method of generating multiple copies of a nucleic acid sequence of interest, said method comprising the steps of:

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(a) hybridizing a first oligonucleotide and a second oligonucleotide to non-overlapping portions of a target polynucleotide, wherein the portion of the target polynucleotide that is hybridizable to the first oligonucleotide is 3' with respect to the portion of the target nucleotide that is hybridizable to the second oligonucleotide, wherein at least one of said oligonucleotides is a composite primer comprising an RNA portion and a DNA portion, and wherein at least one of said oligonucleotides comprises a sequence that is hybridizable to at least one nucleotide of the sequence of interest;

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(b) optionally extending the first oligonucleotide;

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(c) attaching the first oligonucleotide and second oligonucleotide to each other when hybridized to said target polynucleotide to generate an attached oligonucleotide combination product; and

(d) cleaving the RNA portion of the attached oligonucleotide combination product of (c) with an enzyme that cleaves RNA from an RNA/DNA hybrid such that the cleaved oligonucleotide combination product dissociates from the target polynucleotide.

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wherein the attached oligonucleotide combination product is of a size that when the RNA is cleaved from the attached oligonucleotide combination product, the cleaved attached oligonucleotide product dissociates from the target polynucleotide under essentially the same conditions as those for attachment of the oligonucleotides, whereby multiple copies of the sequence of interest are produced.

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17. The method of claim 16, wherein the first oligonucleotide comprises a sequence hybridizable to at least one nucleotide of the sequence of interest.

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18. The method of claim 16, wherein the 3' most nucleotide of the first oligonucleotide is hybridizable to the sequence of interest.

19. The method of claim 16, wherein the second oligonucleotide comprises a sequence hybridizable to at least one nucleotide of the sequence of interest.

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20. The method of claim 16, wherein the 5' most nucleotide of the second oligonucleotide is hybridizable to at least one nucleotide of the sequence of interest.

21. The method of claim 16, wherein the sequence of interest is a single nucleotide base.

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22. The method of claim 16, wherein the sequence of interest is more than a single nucleotide base.

23. The method of claim 16, wherein the first and second oligonucleotide each comprise a sequence hybridizable to at least one nucleotide of the sequence of interest.

5           24. The method of claim 16, wherein the enzyme that cleaves RNA is RNase H.

25. The method of claim 16, wherein the DNA polymerase lacks strand displacement activity.

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26. The method of claim 16, wherein the target polynucleotide is DNA or cDNA.

27. The method of claim 16, wherein the target polynucleotide is amplified.

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28. The method of claim 27, wherein amplification occurs simultaneously with generating multiple copies of a sequence of interest.

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29. The method of claim 27, wherein amplification occurs prior to generating multiple copies of a sequence of interest.

30. The method of claim 16, wherein the attaching is covalent.

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31. The method of claim 30, wherein the covalent attachment is effected by ligase.

32. A method of determining whether a nucleic acid sequence of interest is present or absent in a sample, said method comprising the steps of:

(a) hybridizing a composite primer to a target polynucleotide, wherein the composite primer comprises an RNA portion and a 3' DNA portion, the 3' DNA portion comprising a 3' most nucleotide, such that the 3' most nucleotide of the DNA portion of the composite primer hybridizes from about 1 to about 10 nucleotides from the sequence of interest;

(b) extending the composite primer with DNA polymerase under conditions that permit primer extension, whereby a primer extension product comprising a detectable identifying characteristic is produced if the sequence of interest is present; and

(c) cleaving the RNA portion of the primer extension product of (b), if any, with an enzyme that cleaves RNA from an RNA/DNA hybrid such that the cleaved primer extension product dissociates from the target polynucleotide,

wherein the primer extension product is of a size that when the RNA is cleaved the cleaved primer extension product dissociates from the target polynucleotide under essentially the same conditions as those for primer extension, whereby detection of the cleaved primer extension product comprising the detectable identifying characteristic indicates the presence of the sequence of interest.

33. The method of claim 32, wherein said conditions that permit primer extension comprise at least one terminator deoxyribonucleotide triphosphate or analog thereof.

34. The method of claim 32, wherein said conditions that permit primer extension comprise the absence of a nucleotide that must be provided for primer extension to continue.

35. The method of claim 32, wherein the 3' most nucleotide of the 3' DNA portion of the composite primer hybridizes 1 nucleotide from the sequence of interest.

5 36. The method of claim 32, wherein the 3' most nucleotide of the 3' DNA portion of the composite primer hybridizes 1 nucleotide from the sequence of interest, the sequence of interest is a single nucleotide, and the conditions for primer extension comprise one terminator deoxyribonucleotide triphosphate or analog thereof.

10 37. The method of claim 32, wherein the sequence of interest is a single nucleotide base.

38. The method of claim 32, wherein the sequence of interest is more than a single nucleotide base.

15 39. The method of claim 32, wherein the RNA portion of the composite primer is 5' with respect to the 3' DNA portion.

40. The method of claim 32, wherein the 5' RNA portion is adjacent to the 3' DNA portion.

20 41. The method of claim 32, wherein the enzyme that cleaves RNA is RNase H.

25 42. The method of claim 32, wherein the DNA polymerase lacks strand displacement activity.

43. The method of claim 32, wherein the target polynucleotide is DNA or cDNA.

44. The method of claim 32, wherein the target polynucleotide is amplified.

45. The method of claim 44, wherein amplification occurs simultaneously with generating multiple copies of a sequence of interest.

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46. The method of claim 44, wherein amplification occurs prior to generating multiple copies of a sequence of interest.

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47. The method of claim 32, wherein the target polynucleotide is attached to an analyte.

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48. The method of claim 47, wherein the analyte is selected from the group consisting of a polypeptide, an antibody, an organic molecule and an inorganic molecule.

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49. The method of claim 32, wherein said detectable identifying characteristic is selected from the group consisting of size of the cleaved primer extension product, sequence of the cleaved primer extension product, and detectable signal associated with the cleaved primer extension product.

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50. The method of claim 32, wherein the detectable identifying characteristic comprises the sequence of the cleaved primer extension product, wherein the sequence is detected by hybridizing the cleaved primer extension product with a nucleic acid probe that is hybridizable to the cleaved primer extension product.

51. The method of claim 50, wherein said nucleic acid probe comprises DNA.



52. The method of claim 50, wherein the nucleic acid probe is provided as an array.

5 53. The method of claim 52, wherein the array comprises the probe immobilized on a substrate fabricated from a material selected from the group consisting of paper, glass, plastic, polypropylene, nylon, polyacrylamide, nitrocellulose, silicon, polystyrene, and optical fiber.

10 54. The method of claim 49, wherein said detectable signal is associated with a label on a deoxyribonucleotide triphosphate or analog thereof that is incorporated during primer extension.

15 55. The method of claim 49, wherein said detectable signal is associated with interaction of two labels, wherein one label is on a deoxyribonucleotide triphosphate or analog thereof that is incorporated during primer extension and another label is on a deoxyribonucleotide triphosphate or analog thereof located in the primer portion of the primer extension product.

20 56. A method of determining whether a nucleic acid sequence of interest is present or absent in a sample, said method comprising the steps of:

25 (a) hybridizing a first oligonucleotide and a second oligonucleotide to non-overlapping portions of a target polynucleotide, wherein the portion of the target polynucleotide that is hybridizable to the first oligonucleotide is 3' with respect to the portion of the target nucleotide that is hybridizable to the second oligonucleotide, wherein at least one of said oligonucleotides is a composite primer comprising an RNA portion and a DNA portion, and wherein at least one of said oligonucleotides

comprises a sequence that is hybridizable to at least one nucleotide of the sequence of interest;

(b) optionally extending the first oligonucleotide;

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(c) attaching the first oligonucleotide and second oligonucleotide to each other when hybridized to said target polynucleotide to generate an attached oligonucleotide combination product comprising a detectable identifying characteristic, whereby the attached oligonucleotide combination product is produced if the sequence of interest is present; and

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(d) cleaving the RNA portion of the attached oligonucleotide combination product of (c), if any, with an enzyme that cleaves RNA from an RNA/DNA hybrid such that the cleaved attached oligonucleotide combination product dissociates from the target polynucleotide,

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wherein the attached oligonucleotide combination product is of a size that when the RNA is cleaved the cleaved attached oligonucleotide combination product dissociates from the target polynucleotide under essentially the same conditions as those for attachment of the oligonucleotides, whereby detection of the cleaved attached oligonucleotide combination product comprising the detectable identifying characteristic indicates the presence of the sequence of interest.

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57. The method of claim 56, wherein the first oligonucleotide comprises a sequence hybridizable to at least one nucleotide of the sequence of interest.

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58. The method of claim 56, wherein the 3' most nucleotide of the first oligonucleotide is hybridizable to the sequence of interest.

59. The method of claim 56, wherein the second oligonucleotide comprises a sequence hybridizable to at least one nucleotide of the sequence of interest.

5 60. The method of claim 56, wherein the 5' most nucleotide of the second oligonucleotide is hybridizable to at least one nucleotide of the sequence of interest.

61. The method of claim 56, wherein the sequence of interest is a single nucleotide base.

10 62. The method of claim 56, wherein the sequence of interest is more than a single nucleotide base.

15 63. The method of claim 56, wherein the first and second oligonucleotide each comprise a sequence hybridizable to at least one nucleotide of the sequence of interest.

64. The method of claim 56, wherein the enzyme that cleaves RNA is RNase H.

20 65. The method of claim 56, wherein the DNA polymerase lacks strand displacement activity.

25 66. The method of claim 56, wherein the target polynucleotide is DNA or cDNA.

67. The method of claim 56, wherein the target polynucleotide is amplified.

68. The method of claim 67, wherein amplification occurs simultaneously with generating multiple copies of a sequence of interest.

5 69. The method of claim 67, wherein amplification occurs prior to generating multiple copies of a sequence of interest.

70. The method of claim 56, wherein the target polynucleotide is attached to an analyte.

10 71. The method of claim 61, wherein the analyte is selected from the group consisting of a peptide, an antibody, an organic molecule and an inorganic molecule.

15 72. The method of claim 56, wherein said detectable identifying characteristic is selected from the group consisting of size of the cleaved attached oligonucleotide combination product, sequence of the cleaved attached oligonucleotide combination product, and detectable signal associated with the cleaved attached oligonucleotide combination product.

20 73. The method of claim 56, wherein the detectable identifying characteristic comprises the sequence of the cleaved attached oligonucleotide product, and wherein the sequence is detected by hybridizing the cleaved attached oligonucleotide product with a nucleic acid probe that is hybridizable to the cleaved attached oligonucleotide product.

25 74. The method of claim 73, wherein said nucleic acid probe comprises DNA.

75. The method of claim 73, wherein the nucleic acid probe is provided as a array.

76. The method of claim 75, wherein said array comprises the probe immobilized on a substrate fabricated from a material selected from the group consisting of paper, glass, plastic, polypropylene, nylon, polyacrylamide, nitrocellulose, silicon, polystyrene, and optical fiber.

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77. The method of claim 72, wherein said detectable signal is associated with a label on a deoxyribonucleotide triphosphate or analog thereof that is incorporated during primer extension.

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78. The method of claim 72, wherein said detectable signal is associated with interaction of two labels, wherein one label is located in the first oligonucleotide and a second label is located in the second oligonucleotide.

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79. The method of claim 72, wherein said detectable signal is associated with interaction of two labels, wherein one label is located either the first or second oligonucleotide, and a second label is located on a deoxyribonucleotide triphosphate or analog thereof that is incorporated during primer extension.

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80. The method of claim 56, wherein the attaching is covalent attachment.

81. The method of claim 80, wherein the covalent attachment is effected by ligase.

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82. A method of generating multiple copies of a nucleic acid sequence of interest comprising:

incubating a reaction mixture, said reaction mixture comprising:

(a) a target polynucleotide;

(b) a composite primer that hybridizes to the target polynucleotide, said composite primer comprising an RNA portion and a 3' DNA portion, the 3' DNA portion comprising a 3' most nucleotide, such that the 3' most nucleotide of the 3' DNA portion of the primer hybridizes from about 1 nucleotide to about 10 nucleotides from the sequence of interest;

(c) a DNA polymerase; and

(d) an enzyme that cleaves RNA from an RNA/DNA hybrid,

wherein the incubation is under conditions that permit primer hybridization, primer extension and RNA cleavage, such that a primer extension product is produced, and wherein the primer extension product is of a size such that cleavage of RNA from the primer extension product results in dissociation of the cleaved primer extension product from the target polynucleotide.

83. The method of claim 82, wherein said conditions that permit primer extension comprise at least one terminator deoxyribonucleotide triphosphate or analog thereof.

84. The method of claim 82, wherein said conditions that permit primer extension comprise the absence of a nucleotide that must be provided for primer extension to continue.

85. The method of claim 82, wherein the 3' most nucleotide of the 3' DNA portion of the composite primer hybridizes 1 nucleotide from the sequence of interest.

86. The method of claim 85, wherein the conditions for primer extension comprise one terminator deoxyribonucleotide triphosphate or analog thereof.

5 87. The method of claim 82, wherein the sequence of interest is a single nucleotide base.

88. The method of claim 82, wherein the sequence of interest is more than a single nucleotide base.

10 89. The method of claim 82, wherein the RNA portion of the composite primer is 5' with respect to the 3' DNA portion.

90. The method of claim 82, wherein the 5' RNA portion is adjacent to the 3' DNA portion.

15 91. The method of claim 82, wherein the enzyme that cleaves RNA is RNase H.

20 92. The method of claim 82, wherein the DNA polymerase lacks strand displacement activity.

93. The method of claim 82, wherein the target polynucleotide is DNA or cDNA.

25 94. The method of claim 82, wherein the target polynucleotide is amplified.

95. The method of claim 94, wherein amplification occurs simultaneously with generating multiple copies of a sequence of interest.

96. The method of claim 94, wherein amplification occurs prior to generating multiple copies of a sequence of interest.

5 97. A method of generating multiple copies of a nucleic acid sequence of interest comprising incubating a reaction mixture, said reaction mixture comprising:

(a) a target polynucleotide;

10 (b) a first oligonucleotide and a second oligonucleotide that hybridize to non-overlapping portions of a target polynucleotide, wherein the portion of the target polynucleotide that is hybridizable to the first oligonucleotide is 3' with respect to the portion of the target nucleotide that is hybridizable to the second oligonucleotide, wherein at least one of said oligonucleotides is a composite primer comprising an RNA portion and a DNA portion, and wherein at least one of said oligonucleotides  
15 comprises a sequence that is hybridizable to at least one nucleotide of the sequence of interest;

(c) optionally a DNA polymerase;

20 (d) an enzyme that cleaves RNA from an RNA/DNA hybrid; and

(e) an agent that effects attachment of the first oligonucleotide and second oligonucleotide to each other when said oligonucleotides are hybridized to the target polynucleotide.

25 wherein the incubation is under conditions that permit oligonucleotide hybridization, optionally oligonucleotide extension, RNA cleavage and attachment of the first oligonucleotide and the second oligonucleotide, such that an attached



oligonucleotide combination product is produced, and wherein the attached oligonucleotide combination product is of a size such that cleavage of RNA from the attached oligonucleotide combination product results in dissociation of the cleaved attached oligonucleotide product.

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98. The method of claim 97, wherein the first oligonucleotide comprises a sequence hybridizable to at least one nucleotide of the sequence of interest.

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99. The method of claim 97, wherein the 3' most nucleotide of the first oligonucleotide is hybridizable to the sequence of interest.

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100. The method of claim 97, wherein the second oligonucleotide comprises a sequence hybridizable to at least one nucleotide of the sequence of interest.

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101. The method of claim 97, wherein the 5' most nucleotide of the second oligonucleotide is hybridizable to at least one nucleotide of the sequence of interest.

102. The method of claim 97, wherein the sequence of interest is a single nucleotide base.

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103. The method of claim 97, wherein the sequence of interest is more than a single nucleotide base.

104. The method of claim 97, wherein the first and second oligonucleotide each comprise a sequence hybridizable to at least one nucleotide of the sequence of interest.

105. The method of claim 97, wherein the enzyme that cleaves RNA is RNase H.

5 106. The method of claim 97, wherein the DNA polymerase lacks strand displacement activity.

107. The method of claim 97, wherein the target polynucleotide is DNA or cDNA.

10 108. The method of claim 97, wherein the target polynucleotide is amplified.

109. The method of claim 108, wherein amplification occurs simultaneously with generating multiple copies of a sequence of interest.  
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110. The method of claim 108, wherein amplification occurs prior to generating multiple copies of a sequence of interest.

20 111. The method of claim 97, wherein the attaching is covalent.

112. The method of claim 111, wherein the covalent attachment is effected by ligase.

113. A method of determining whether a nucleic acid sequence of interest is present or absent in a sample comprising incubating a reaction mixture, said  
25 reaction mixture comprising:

(a) a target polynucleotide;

(b) a composite primer that hybridizes to the target polynucleotide, said composite primer comprising an RNA portion and a 3' DNA portion, the 3' DNA portion comprising a 3' most nucleotide, such that the 3' most nucleotide of the 3' DNA portion of the primer hybridizes from about 1 nucleotide to about 10 nucleotides from the sequence of interest;

(c ) a DNA polymerase; and

(d) an enzyme that cleaves RNA from an RNA/DNA hybrid,

wherein the incubation is under conditions that permit primer hybridization, primer extension to generate a primer extension product comprising a detectable identifying characteristic, and RNA cleavage, such that the primer extension product comprising a detectable identifying characteristic is produced, and wherein the primer extension product is of a size such that cleavage of RNA from the primer extension product results in dissociation of the cleaved primer extension product from the target polynucleotide, whereby detection of the cleaved primer extension product comprising the detectable identifying characteristic indicates presence of the nucleotide sequence of interest.

114. The method of claim 113, wherein said conditions that permit primer extension comprise at least one terminator deoxyribonucleotide triphosphate or analog thereof.

115. The method of claim 113, wherein said conditions that permit primer extension comprise the absence of a nucleotide that must be provided for primer extension to continue.

116. The method of claim 113, wherein the 3' most nucleotide of the 3' DNA portion of the composite primer hybridizes 1 nucleotide from the sequence of interest.

5 117. The method of claim 113, wherein the 3' most nucleotide of the 3' DNA portion of the composite primer hybridizes 1 nucleotide from the sequence of interest, the sequence of interest is a single nucleotide, and the conditions for primer extension comprise one terminator deoxyribonucleotide triphosphate or analog thereof.

10 118. The method of claim 113, wherein the sequence of interest is a single nucleotide base.

15 119. The method of claim 113, wherein the sequence of interest is more than a single nucleotide base.

120. The method of claim 113, wherein the RNA portion of the composite primer is 5' with respect to the 3' DNA portion.

20 121. The method of claim 113, wherein the 5' RNA portion is adjacent to the 3' DNA portion.

122. The method of claim 113, wherein the enzyme that cleaves RNA is RNase H.

25 123. The method of claim 113, wherein the DNA polymerase lacks strand displacement activity.

124. The method of claim 113, wherein the target polynucleotide is DNA or cDNA.

5 125. The method of claim 113, wherein the target polynucleotide is amplified.

126. The method of claim 125, wherein amplification occurs simultaneously with generating multiple copies of a sequence of interest.

10 127. The method of claim 125, wherein amplification occurs prior to generating multiple copies of a sequence of interest.

128. The method of claim 113, wherein the target polynucleotide is attached to an analyte.  
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129. The method of claim 128, wherein the analyte is selected from the group consisting of a polypeptide, an antibody, an organic molecule and an inorganic molecule.

20 130. The method of claim 113, wherein said detectable identifying characteristic is selected from the group consisting of size of the cleaved primer extension product, sequence of the cleaved primer extension product, and detectable signal associated with the cleaved primer extension product.

25 131. The method of claim 113, wherein the detectable identifying characteristic comprises the sequence of the cleaved primer extension product, wherein the sequence is detected by hybridizing the cleaved primer extension product with a nucleic acid probe that is hybridizable to the cleaved primer extension product.

132. The method of claim 131, wherein said nucleic acid probe comprises DNA.

133. The method of claim 131, wherein the nucleic acid probe is immobilized on a surface.

134. The method of claim 133, wherein the surface comprises a substrate fabricated from a material selected from the group consisting of paper, glass, plastic, polypropylene, nylon, polyacrylamide, nitrocellulose, silicon, polystyrene, and optical fiber

135. The method of claim 130, wherein said detectable signal is associated with a label on a deoxyribonucleotide triphosphate or analog thereof that is incorporated during primer extension.

136. The method of claim 130, wherein said detectable signal is associated with interaction of two labels, wherein one label is on a deoxyribonucleotide triphosphate or analog thereof that is incorporated during primer extension and another label is on a deoxyribonucleotide triphosphate or analog thereof located in the primer portion of the primer extension product.

137. A method of determining whether a nucleic acid sequence of interest is present or absent in a sample comprising incubating a reaction mixture, said reaction mixture comprising:

(a) a target polynucleotide;

(b) a first oligonucleotide and a second oligonucleotide that hybridize to non-overlapping portions of a target polynucleotide, wherein the portion of the target polynucleotide that is hybridizable to the first oligonucleotide is 3' with respect to the portion of the target nucleotide that is hybridizable to the second oligonucleotide,  
5 wherein at least one of said oligonucleotides is a composite primer comprising an RNA portion and a DNA portion, and wherein at least one of said oligonucleotides comprises a sequence that is hybridizable to at least one nucleotide of the sequence of interest;

10 (c) optionally a DNA polymerase;

(d) an enzyme that cleaves RNA from an RNA/DNA hybrid; and

(e) an agent that effects attachment of the first oligonucleotide and second  
15 oligonucleotide to each other when said oligonucleotides are hybridized to the target polynucleotide,

wherein the incubation is under conditions that permit oligonucleotide hybridization, optionally oligonucleotide extension, RNA cleavage and attachment of  
20 the first oligonucleotide and the second oligonucleotide, such that an attached oligonucleotide combination product comprising a detectable identifying characteristic is produced, and wherein the attached oligonucleotide combination product is of a size such cleavage of the RNA from the attached oligonucleotide combination product results in dissociation of the cleaved attached oligonucleotide  
25 combination product from the target polynucleotide, whereby detection of the cleaved attached oligonucleotide combination product comprising the detectable identifying characteristic indicates presence of the nucleotide sequence of interest.

138. The method of claim 137, wherein the first oligonucleotide comprises a sequence hybridizable to at least one nucleotide of the sequence of interest.

5 139. The method of claim 137, wherein the 3' most nucleotide of the first oligonucleotide is hybridizable to the sequence of interest.

140. The method of claim 137, wherein the second oligonucleotide comprises a sequence hybridizable to at least one nucleotide of the sequence of interest.

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141. The method of claim 137, wherein the 5' most nucleotide of the second oligonucleotide is hybridizable to at least one nucleotide of the sequence of interest.

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142. The method of claim 137, wherein the sequence of interest is a single nucleotide base.

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143. The method of claim 137, wherein the sequence of interest is more than a single nucleotide base.

144. The method of claim 137, wherein the first and second oligonucleotide each comprise a sequence hybridizable to at least one nucleotide of the sequence of interest.

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145. The method of claim 137, wherein the enzyme that cleaves RNA is RNase H.



146. The method of claim 137, wherein the DNA polymerase lacks strand displacement activity.

5 147. The method of claim 137, wherein the target polynucleotide is DNA or cDNA.

148. The method of claim 137, wherein the target polynucleotide is amplified.

10 149. The method of claim 148, wherein amplification occurs simultaneously with generating multiple copies of a sequence of interest.

15 150. The method of claim 148, wherein amplification occurs prior to generating multiple copies of a sequence of interest.

151. The method of claim 137, wherein the target polynucleotide is attached to an analyte.

20 152. The method of claim 151, wherein the analyte is selected from the group consisting of a peptide, an antibody, an organic molecule and an inorganic molecule.

25 153. The method of claim 137, wherein said detectable identifying characteristic is selected from the group consisting of size of the cleaved attached oligonucleotide combination product, sequence of the cleaved attached oligonucleotide combination product, and detectable signal associated with the cleaved attached oligonucleotide combination product.

154. The method of claim 137, wherein the detectable identifying characteristic comprises the sequence of the cleaved attached oligonucleotide product, and wherein the sequence is detected by hybridizing the cleaved attached oligonucleotide product with a nucleic acid probe that is hybridizable to the cleaved attached oligonucleotide product.

155. The method of claim 154, wherein said nucleic acid probe comprises DNA.

156. The method of claim 154, wherein the nucleic acid probe is immobilized on a surface.

157. The method of claim 156, wherein said surface comprises a substrate fabricated from a material selected from the group consisting of paper, glass, plastic, polypropylene, nylon, polyacrylamide, nitrocellulose, silicon, polystyrene, and optical fiber.

158. The method of claim 153, wherein said detectable signal is associated with a label on a deoxyribonucleotide triphosphate or analog thereof that is incorporated during primer extension.

159. The method of claim 153, wherein said detectable signal is associated with interaction of two labels, wherein one label is located in the first oligonucleotide and a second label is located in the second oligonucleotide.

160. The method of claim 153, wherein said detectable signal is associated with interaction of two labels, wherein one label is located either the first or second

oligonucleotide, and a second label is located on a deoxyribonucleotide triphosphate or analog thereof that is incorporated during primer extension.

5           161.   The method of claim 137, wherein the attaching is covalent attachment.

          162.   The method of claim 161, wherein the covalent attachment is effected by ligase.

10           163.   The method of claims 32 or 113, wherein said method comprises determining whether two or more sequences of interest are present or absent in a sample, said method comprising using one or more different composite primers, wherein the detectable identifying characteristics of the cleaved primer extension products corresponding to two different sequences of interest are different from each  
15           other.

          164.   The method of claim 163, wherein said method comprises at least two different composite primers.

20           165.   The method of claim 163, wherein at least one of the sequences of interest is a variant of the sequence of interest.

          166.   The method of 56 or 137, wherein said method comprises determining whether two or more different sequences of interest are present or absent in a sample,  
25           said method using a two or more sets of first and second oligonucleotides, wherein the detectable identifying characteristics of the cleaved oligonucleotide attachment products corresponding to two or more different sequences of interest are different from each other.

167. The method of claim 166, wherein at least one of the sequences of interest is a variant of the sequence of interest.

168. A method for identifying an altered sequence of interest in a sample comprising incubating a reaction mixture, said reaction mixture comprising:

(a) a target polynucleotide;

(b) a composite primer that hybridizes to the target polynucleotide, said composite primer comprising an RNA portion and a 3' DNA portion, the 3' DNA portion comprising a 3' most nucleotide, such that the 3' most nucleotide of the 3' DNA portion of the primer hybridizes from about 1 nucleotide to about 10 nucleotides from the altered sequence of interest;

(c ) a DNA polymerase; and

(d) an enzyme that cleaves RNA from an RNA/DNA hybrid,

wherein the incubation is under conditions that permit primer hybridization and primer extension to generate a primer extension product comprising a detectable identifying characteristic, and RNA cleavage, such that a primer extension product comprising a detectable identifying characteristic is produced, and

wherein the primer extension product is of a size that when RNA is cleaved from the primer extension product, the cleaved primer extension product dissociates from the target polynucleotide, whereby the cleaved primer extension product is characterized to identify the altered sequence of interest.

169. The method of claim 168, wherein the cleaved primer extension product produced from the target polynucleotide comprises a different detectable identifying characteristic as compared with a detectable identifying characteristic of a cleaved primer extension product produced from a reference template containing the sequence of interest, wherein the characterization comprises comparing the detectable identifying characteristics.

170. A method of identifying an altered sequence of interest in a sample, said method comprising incubating a reaction mixture, said reaction mixture comprising:

(a) a target polynucleotide;

(b) a composite primer that hybridizes to the target polynucleotide, said composite primer comprising an RNA portion and a 3' DNA portion, the 3' DNA portion comprising a 3' most nucleotide, such that the 3' most nucleotide of the 3' DNA portion of the primer hybridizes from about 1 nucleotide to about 10 nucleotides from the altered sequence of interest;

(c ) a DNA polymerase; and

(d) an enzyme that cleaves RNA from an RNA/DNA hybrid,

wherein the incubation is under conditions that permit primer hybridization, and primer extension to generate a primer extension product comprising a detectable identifying characteristic, and RNA cleavage, such that the primer extension product comprising a detectable identifying characteristic is produced, and

wherein the primer extension product is of a size that when RNA is cleaved from the primer extension product, the cleaved primer extension product dissociates from the target polynucleotide, and

5            wherein production of detectably fewer cleaved primer extension products from the target as compared to the amount of cleaved primer extension products produced from a reference template comprising the sequence of interest indicates that the target polynucleotide contains an altered sequence of interest.

10            171. The method of claim 170, further comprising characterizing the sequence of the template polynucleotide about 1 to about 10 nucleotides 5' from the sequence hybridizable to the composite primer.

15            172. A method of identifying an altered sequence of interest in a sample, said method comprising incubating a reaction mixture, said reaction mixture comprising:

(a) a target polynucleotide;

20            (b) a first oligonucleotide and a second oligonucleotide that hybridize to non-overlapping portions of a target polynucleotide, wherein the portion of the target polynucleotide that is hybridizable to the first oligonucleotide is 3' with respect to the portion of the target nucleotide that is hybridizable to the second oligonucleotide, wherein at least one of said oligonucleotides is a composite primer comprising an  
25            RNA portion and a DNA portion, and wherein at least one of said oligonucleotides comprises a sequence that is hybridizable to at least one nucleotide of the sequence of interest;

(c) optionally a DNA polymerase;

(d) an enzyme that cleaves RNA from an RNA/DNA hybrid; and

5           (e) an agent that effects attachment of the first oligonucleotide and second oligonucleotide to each other when said oligonucleotides are hybridized to the target polynucleotide.

          wherein the incubation is under conditions that permit oligonucleotide  
10   hybridization, optionally oligonucleotide extension, RNA cleavage and attachment of the first oligonucleotide and the second oligonucleotide, such that an attached oligonucleotide combination product comprising a detectable identifying characteristic is produced, and wherein the attached oligonucleotide combination product is of a size that when RNA is cleaved from the attached oligonucleotide  
15   combination product, the cleaved attached oligonucleotide combination product dissociates from the target polynucleotide.

          wherein production of detectably fewer cleaved oligonucleotide attachment products from the target as compared to the amount of cleaved oligonucleotide  
20   attachment products produced from a reference template comprising the sequence of interest indicates that the target polynucleotide contains an altered sequence of interest.

173.   The method of claim 172, further comprising characterizing sequence  
25   of the target polynucleotide.

174.   A kit for generation of multiple copies of a sequence of interest, comprising a composite primer comprising a 3' DNA portion and an RNA portion.

and instructions for a method for generating multiple copies, said method comprising incubating a reaction mixture, said reaction mixture comprising:

(a) a target polynucleotide;

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(b) a composite primer that hybridizes to the target polynucleotide, said composite primer comprising an RNA portion and a 3' DNA portion, the 3' DNA portion comprising a 3' most nucleotide, such that the 3' most nucleotide of the 3' DNA portion of the primer hybridizes from about 1 nucleotide to about 10 nucleotides from the sequence of interest;

10

(c ) a DNA polymerase; and

(d) an enzyme that cleaves RNA from an RNA/DNA hybrid,

15

wherein the incubation is under conditions that permit primer hybridization, primer extension and RNA cleavage, such that a primer extension product is produced, and wherein the primer extension product is of a size such that cleavage of RNA from the primer extension product results in dissociation of the cleaved primer extension product, whereby multiple copies are generated.

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175. The kit of claim 174, further comprising terminator deoxyribonucleotide triphosphates.

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176. The kit of claim 174 or 175, further comprising at least one but not all four types of deoxyribonucleotide triphosphates.



177. A kit for determining whether a sequence of interest is present or absent in a sample, comprising a composite primer comprising a 3' DNA portion and an RNA portion, and instructions for a method of determining whether a sequence of interest is present or absent in a sample, said method comprising incubating a reaction mixture, said reaction mixture comprising:

(a) a target polynucleotide;

(b) a composite primer that hybridizes to the target polynucleotide, said composite primer comprising an RNA portion and a 3' DNA portion, the 3' DNA portion comprising a 3' most nucleotide, such that the 3' most nucleotide of the 3' DNA portion of the primer hybridizes from about 1 nucleotide to about 10 nucleotides from the sequence of interest;

(c ) a DNA polymerase; and

(d) an enzyme that cleaves RNA from an RNA/DNA hybrid,

wherein the incubation is under conditions that permit primer hybridization, primer extension to generate a primer extension product comprising a detectable identifying characteristic, and RNA cleavage, such that the primer extension product comprising a detectable identifying characteristic is produced, and wherein the primer extension product is of a size that when RNA is cleaved from the primer extension product, the cleaved primer extension dissociates from the target polynucleotide, whereby detection of the cleaved primer extension product comprising the detectable identifying characteristic indicates presence of the nucleotide sequence of interest.

178. A kit for generation of multiple copies of a sequence of interest, comprising a first oligonucleotide and a second oligonucleotide, wherein at least one oligonucleotide is a composite primer, wherein the two oligonucleotides hybridize to non-overlapping portions of a target polynucleotide, wherein the portion of the target polynucleotide that is hybridizable to the first oligonucleotide is 3' with respect to the portion of the target nucleotide that is hybridizable to the second oligonucleotide, and wherein the oligonucleotides singly or in combination comprise at least one nucleotide of the sequence of interest; and an agent that effects attachment of said first oligonucleotide and second oligonucleotide to each other when said oligonucleotides are hybridized to the target polynucleotide.

179. The kit of claim 178, further comprising instructions for a method of generating multiple copies of a sequence of interest, said method comprising incubating a reaction mixture, said reaction mixture comprising:

(a) a target polynucleotide;

(b) a first oligonucleotide and a second oligonucleotide that hybridize to non-overlapping portions of a target polynucleotide, wherein the portion of the target polynucleotide that is hybridizable to the first oligonucleotide is 3' with respect to the portion of the target nucleotide that is hybridizable to the second oligonucleotide, wherein at least one of said oligonucleotides is a composite primer comprising an RNA portion and a DNA portion, and wherein at least one of said oligonucleotides comprises a sequence that is hybridizable to at least one nucleotide of the sequence of interest;

(c) optionally a DNA polymerase;

(d) an enzyme that cleaves RNA from an RNA/DNA hybrid; and

(e) an agent that effects attachment of the first oligonucleotide and second oligonucleotide to each other when said oligonucleotides are hybridized to the target polynucleotide,

wherein the incubation is under conditions that permit oligonucleotide hybridization, optionally oligonucleotide extension, RNA cleavage and attachment of the first oligonucleotide and the second oligonucleotide, such that an attached oligonucleotide combination product is produced, and wherein the attached oligonucleotide combination product is of a size such that cleavage of RNA results in dissociation of the cleaved attached oligonucleotide product.

180. A kit for determining whether a sequence of interest is present or absent in a sample, comprising a first oligonucleotide and a second oligonucleotide, wherein at least one oligonucleotide is a composite primer, wherein the two oligonucleotides hybridize to non-overlapping portions of a target polynucleotide, wherein the portion of the target polynucleotide that is hybridizable to the first oligonucleotide is 3' with respect to the portion of the target nucleotide that is hybridizable to the second oligonucleotide, and wherein the oligonucleotides singly or in combination comprise at least one nucleotide of the sequence of interest; and an agent that effects attachment of said first oligonucleotide and second oligonucleotide to each other when said oligonucleotides are hybridized to the target polynucleotide.

181. The kit of claim 180, further comprising instructions for a method of determining whether a sequence of interest is present or absent in a sample, said method comprising incubating a reaction mixture, said reaction mixture comprising:

(a) a target polynucleotide;

(b) a first oligonucleotide and a second oligonucleotide that hybridize to non-overlapping portions of a target polynucleotide, wherein the portion of the target polynucleotide that is hybridizable to the first oligonucleotide is 3' with respect to the portion of the target nucleotide that is hybridizable to the second oligonucleotide, wherein at least one of said oligonucleotides is a composite primer comprising an RNA portion and a DNA portion, and wherein at least one of said oligonucleotides comprises a sequence that is hybridizable to at least one nucleotide of the sequence of interest;

(c) optionally a DNA polymerase;

(d) an enzyme that cleaves RNA from an RNA/DNA hybrid; and

(e) an agent that effects attachment of the first oligonucleotide and second oligonucleotide to each other when said oligonucleotides are hybridized to the target polynucleotide,

wherein the incubation is under conditions that permit oligonucleotide hybridization, optionally oligonucleotide extension, RNA cleavage and attachment of the first oligonucleotide and the second oligonucleotide, such that an attached oligonucleotide combination product comprising a detectable identifying characteristic is produced, and wherein the attached oligonucleotide combination product is of a size such that cleavage of RNA from the attached oligonucleotide combination product results in dissociation of the cleaved attached oligonucleotide product from the target polynucleotide, whereby detection of the cleaved primer

extension product comprising the detectable identifying characteristic indicates presence of the nucleotide sequence of interest.

5           182.   The kit of claim 180 or 181, wherein the agent that effects attachment of said first oligonucleotide and second oligonucleotide is ligase.

          183.   The kit of claim 180, further comprising an enzyme that cleaves RNA from a RNA/DNA hybrid.

10           184.   The kit of claim 183, wherein said enzyme is RNase H.

          185.   A reaction mixture comprising (a) a target polynucleotide; (b) a first oligonucleotide and a second oligonucleotide, wherein at least one oligonucleotide is a composite primer, wherein the two oligonucleotides hybridize to non-overlapping  
15           portions of the target polynucleotide, wherein the portion of the target polynucleotide that is hybridizable to the first oligonucleotide is 3' with respect to the portion of the target nucleotide that is hybridizable to the second oligonucleotide, and wherein the oligonucleotides singly comprise at least one nucleotide of the sequence of interest, or in combination comprise at least a portion of the sequence of interest; (c)  
20           optionally DNA polymerase; and (d) an agent that effects covalent attachment of the first oligonucleotide and second oligonucleotide to each other when the oligonucleotides are hybridized to the target polynucleotide.